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CONTRACTING ORGANIZATION:

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13. SUPPLEMENTARY NOTES					
14. ABSTRACT The purpose of this project is to examine how activation of two cellular defense mechanisms mediated by heat shock transcription factor 1 (HSF1) and glucocorticoid receptor (GR) is associated with heat tolerance and heat acclimation. In this first annual period, our major effort focused on examining effects of heat acclimation on activation of the two systems involving HSF1 and GR in cultured muscle cells. Based on our preliminary data, heat-acclimatized cells showed greater resistance against heat stress compared to unacclimatized ones. Heat-acclimatized cells were associated with significant translocation of both HSF1 and GR from the cytosol to nucleus and mitochondria, which was not observed in unacclimatized ones. We also found that heat-induced damage developed concurrently with mitochondrial dysfunction in muscle cells. Inhibition of dynamin-like protein 1 prevented mitochondrial fragmentation and improved viability of muscle cells during heat exposure. These preliminary results suggest that HSF1 and GR systems are involved in HA and mitochondrial integrity is central to resistance of muscle cells against heat-induced injury.					
15. SUBJECT TERMS heat adaptation, heat tolerance, skeletal muscle, C2C12, myotube, in vitro					
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## 1. Introduction

The cellular defense mechanisms mediated by heat shock factor 1 (HSF1) and glucocorticoid receptor (GR) are known to provide primary protection against immediate and prolonged stress. The purpose of this project is to examine the roles of the two systems involving HSF1 and GR in the regulation of heat tolerance and heat acclimation (HA), which remain poorly understood. We propose to assess effects of heat exposure and HA on activation of HSF1-GR systems in our cultured cell model of heat injury and mouse model of heat intolerance as well as in their normal counterparts. Findings from these studies should increase our understanding of potential mechanisms for heat intolerance and provide information useful for future research in heat injury prevention.

## 2. Keywords

hyperthermia, heat shock, heat injury, heat adaptation, rodent, skeletal muscle, C2C12, myotube, hsp, inflammation, cytokines, oxidative stress

## 3. Accomplishments

### What were the major goals of the project?

The major goal of this project is to determine how activation of two stress response systems (HSF1 and GR) is associated with differences in heat tolerance and HA under *in vitro* and *in vivo* conditions. As proposed, we will accomplish three tasks: 1) to examine the effects of HA on cultured cells (August 15, 2014 – August 15, 2015); 2) to examine the relationship between activation of cellular HSF-GR and heat stress in heat-tolerant (TOL) versus -intolerant (INT) mice (August 15, 2014 – August 15, 2016); and 3) to examine the relationship between HSF-GR homeostasis and heat stress responses in TOL versus INT mice following HA (November 15, 2015 – August 15, 2017).

### What opportunities for training and professional development did the project provide?

Nothing to Report

### How were the results disseminated to communities of interest?

Nothing to Report

### What was accomplished under these goals?

The project has made good progress this first annual period. As proposed, our effort was made primarily toward conducting the experiments of Tasks 1 and 2. Specifically, we accomplished the following:

- 1) Task 1:
  - a. Established effective heat shock and HA protocols for the proposed cell studies;
  - b. Tested effective assessments of cell viability against heat stress;
  - c. Validated detection of intracellular HSF1 and GR
- 2) Task 2: Conducted heat stress experiments in 46 TOL or INT mice;
- 3) Task 3: Established an effective HA protocol for the proposed animal studies

Although preliminary, the *in vitro* data (Task 1) reveal a few interesting findings. First, we found that heat stress is associated with dynamin-like protein 1-dependent mitochondrial fragmentation and dysfunction in muscle cells, suggesting that the morphological and functional integrity of mitochondria plays a role in heat tolerance of muscle cells (see Appendix). Second, our recent data indicate that HSF1 (Figure 1) and GR (Figure 2) are indeed translocated from the cytosol to mitochondria and nuclei in heat-acclimatized muscle cells. Additional tests will be conducted to confirm activation of HSF1-GR in these muscle cells by HA.

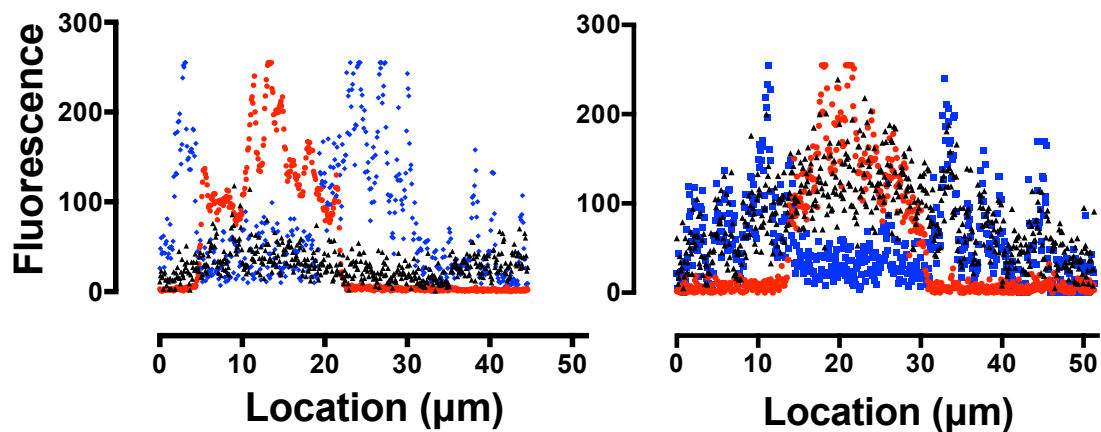


Figure 1. Distribution of HSF1 in C2C12 cells that were incubated at 37 °C (left) or exposed to 39.3 °C for 3 hrs per day (right) for 3 days. Locations of HSF1 (▼), nuclei (●) and mitochondria (■) were calculated relative to a common reference point in each cell. Nuclei and mitochondria were labeled with DAPI and mitoTracker-Red. HSF1 were labeled with Alex 488-conjugated HSF1 antibody. The HSF1 nuclear and mitochondrial colocalizations were greater in heat-acclimatized cells (right) than in controls (left).

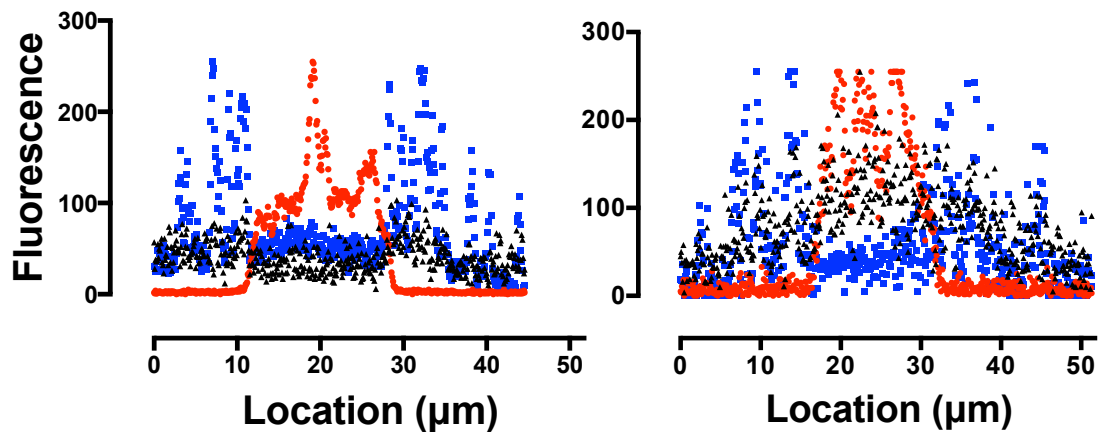


Figure 2. Distribution of GR in C2C12 cells that were incubated at 37 °C (left) or exposed to 39.3 °C for 3 hrs per day (right) for 3 days. Locations of GR (▼), nuclei (●) and mitochondria (■) were calculated relative to a common reference point in each cell. GR were labeled with Alex 488-conjugated GR antibody. The GR nuclear and mitochondrial colocalizations were greater in heat-acclimatized cells (right) than in controls (left).

**What do you plan to do during the next reporting period to accomplish the goals and objectives?**

We will complete Tasks 1 studies and continue the experiments for Tasks 2 and 3. We should have sufficient data that allow us to determine: 1) whether mitochondrial dynamics contribute to heat tolerance and HA; 2) how HSF and GR translocate (activate) from the cytosol to mitochondria and nucleus of muscle cells following heat exposure or HA; and 3) how HSF and GR translocate from the cytosolic to mitochondrial and nuclear fractions in tissues of TOL and INT mice following heat shock.

#### **4. Impact**

**What was the impact on the development of the principal discipline(s) of the project?**

Nothing to Report

**What was the impact on other disciplines?**

Nothing to Report

**What was the impact on technology transfer?**

Nothing to Report

**What was the impact on society beyond science and technology?**

Nothing to Report

#### **5. Changes/Problems**

We experienced delays in fulfilling the proposed positions (one full-time and one part-time) for this project. The full-time scientist was hired during the second quarter of this project (late November 2014). We were unable to fulfill the part-time position, but instead hired a college student to work full-time for two months during this year. The project is progressing as planned.

#### **6. Products**

Poster presentation at Uniformed Services University Research Days, May 2015

Tianzheng Yu, Yifan Chen: "Inhibition of mitochondrial fission reduces heat-induced damage in skeletal muscle cells"

(see Appendix)

#### **7. Participants & Other Collaborating Organizations**

**What individuals have worked on the project?**

Name: Yifan Chen

Project Role: PI

Researcher Identifier (ORCID ID): 0000-0003-1388-9200

Nearest person month worked: 4

Contribution to Project: performed work in project management and provided assistance in experiments.

Name: Tianzheng Yu

Project Role: Senior Scientist

Researcher Identifier (e.g. ORCID ID): N/A

Nearest person month worked: 9

Contribution to Project: conducted the cell culture and animal experiments.

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to Report

**What other organizations were involved as partners?**

Nothing to Report

#### **8. Special Reporting Requirements**

None



# Inhibition of mitochondrial fission reduces heat-induced damage in skeletal muscle cells

Tianzheng Yu and Yifan Chen

Consortium for Health and Military Performance, Department of Military and Emergency Medicine, USU, Bethesda, Maryland



## 9. Appendix

Poster presentation at Uniformed Services University Research Days, May 2015

### INTRODUCTION

The ability of skeletal muscle cells to maintain homeostasis under stress is critical for injury prevention. Mitochondria are highly dynamic organelles and contribute to rapid cellular adaptation to metabolic and environmental stress by shifting between tubular and fragmented network-like morphologies. If fission, which is mediated by dynamin like protein 1 (DLP1), outweighs fusion, mitochondria vesiculate.



Alterations in mitochondrial morphology may lead to mitochondrial dysfunction and ultimately affect cell integrity or resistance against stress-induced insults. How the mitochondria in skeletal muscle change morphologically in response to heat stress remains poorly understood.

### OBJECTIVE

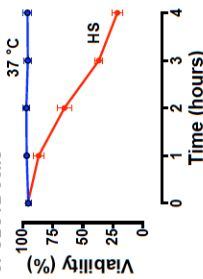
To investigate whether mitochondrial morphology plays a role in thermal tolerance in mouse skeletal muscle *in vitro*

### METHODS

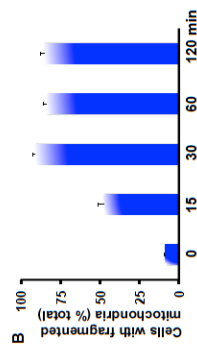
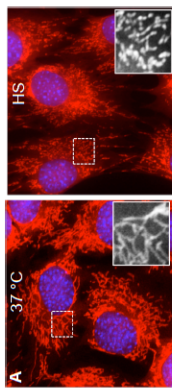
Mouse C2C12 myoblast cells were incubated at 37°C or 43 - 45 °C (heat shock, HS). Cell viability was measured by trypan blue exclusion test using bio-rad cell counter. Mitochondrial morphology were visualized using MitoTracker Red. DLP1 was assessed by immunoblotting and immunofluorescence. Mitochondrial membrane potential, the proton gradient critical for maintaining the physiological function of the respiratory chain to generate ATP, were measured using cell-permeant cationic fluorescent dye TMRE.

### RESULTS

#### 1. Viability of C2C12 cells

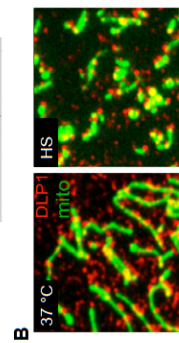
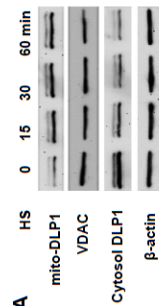


#### 2. Mitochondrial morphology



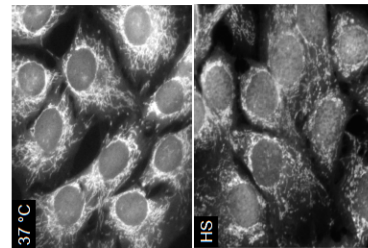
Mitochondrial morphology in C2C12 cells exposed to 37°C or HS (A). Percentage of cells with fragmented mitochondria (B)

#### 3. DLP1 in mitochondria and cytosol



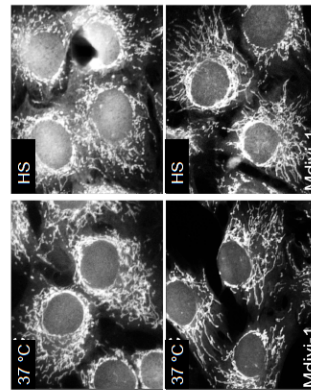
Representative immunoblot (A) and immunofluorescence image (B) of DLP1 in mitochondria and cytosol of C2C12 cells

#### 4. Mitochondrial membrane potential



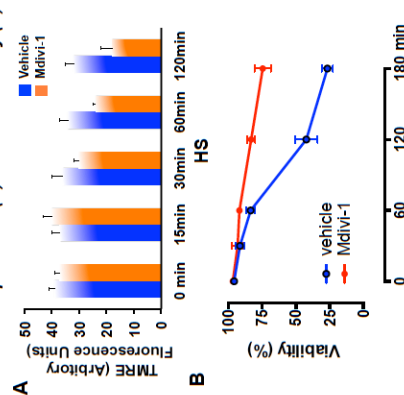
Representative fluorescence images of mitochondria in C2C12 cells loaded with TMRE, an indicator of membrane potential

#### 5. Inhibition of DLP1 on mitochondrial morphology



Mitochondrial morphology in C2C12 cells pretreated with vehicle (top) or Mdivi-1 (bottom), an inhibitor of DLP1

#### 6. Inhibition of DLP1 on mitochondrial membrane potential (A) and cell viability (B)



### SUMMARY

- HS reduced viability and caused mitochondrial fragmentation and translocation of DLP1 from cytosol to mitochondria in muscle cells
- HS caused reductions in mitochondrial membrane potential
- Inhibition of DLP1 reduced HS-induced effects on mitochondrial morphology and membrane potential and improved heat resistance in muscle cells

### CONCLUSIONS

HS causes DLP1-dependent mitochondrial fragmentation and dysfunction in muscle cells. The morphological and functional integrity of mitochondria plays a role in heat tolerance of muscle cells

### ACKNOWLEDGEMENT

This work was supported by Congressionally Directed Medical Research Programs W81XWH-14-2-0133.